UNIVERSITY OF CALIFORNIA PUBLICATIONS

IN

AGRICULTURAL SCIENCES

EDITORS

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VOLUME 1
WITH 19 PLATES

UNIVERSITY OF CALIFORNIA PRESS
BERKELEY
1912-1917

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Vol. 1, No. 1, pp. 1-20 Issued October 15, 1912

THE DISTRIBUTION AND ACTIVITIES OF BACTERIA IN SOILS OF THE ARID REGIONS*

BY
CHARLES B. LIPMAN

INTRODUCTION

The student of soils in the humid region, when for the first time exploring soils in the arid region, is invariably struck with the extraordinary depth of the latter as against the very shallow nature of the former. Taken by and large, and excepting the faulty soils, including those underlaid at no great depth by stiff clay, coarse gravel, hardpan, or original rock, respectively, the soils of the arid region very commonly show a depth of at least eight to ten feet, and, when viewed in section, exhibit such a striking uniformity in texture and color as to attach to this unusual condition, in the mind of the observer, a certain marked practical and scientific interest. The full significance to crops of the arid region of this extraordinary condition in our soils was first realized and pointed out by Hilgard and was made the subject, by him and Loughridge, of a comprehensive investigation on the "soil-columns" of California, a large part of which is completed, but some of which is still in progress. The study of the soil-columns of California comprised what might be looked upon as a very thorough partial soil survey of California. It was the intention of the investigators above named, at the inception of the work, to obtain columns of soil representing depths of twelve feet, including a sample for every foot

* Read before the Society of American Bacteriologists, Washington, D. C., December 27, 1911.

in depth, and to obtain a knowledge of the chemical constitution and the texture of the soils by making systematic chemical and mechanical analyses of all the samples thus collected. The information thus obtained in the several years in which the soil-columns were studied by Hilgard and Loughridge and the large number of types of soils considered, along with the most striking circumstance of the depths to which plant root-systems of the arid regions penetrated, led Hilgard to believe that the striking chemical and mechanical differences between the soils of the arid and humid regions, as well as the differences in the development of the root-systems in these regions, respectively, might find a parallel also in a difference between the bacterial flora at various depths in the soil. It was this belief on Hilgard's part and his valuation thereof as being of exceeding scientific interest as well as practical value, that led to the association with him something over three years ago of the writer, and it was then that I undertook, among other biological problems in soils, a study of the nitrogen-transforming and nitrogen-fixing bacteria in the different layers of soils in the arid region. This study, while it has progressed considerably, is still in the first stage of its development and the complete results thereof are intended ultimately to be combined with the mechanical and chemical analyses of these soils in a comprehensive report on the whole work. For the purposes of this paper, it is sufficient to give a resumé of some of the important results obtained in these investigations with an account of the methods employed in the work, so that it may serve as a preliminary communication on the subject and bring out certain striking facts with reference to the distribution of bacteria in California soils.

METHODS EMPLOYED IN THESE INVESTIGATIONS

One of the most difficult problems in connection with these investigations was to find a method for the collection of soil samples at the several depths which would fairly represent the actual conditions which obtain there, so far as the bacterial flora are concerned. Our first attempts in this direction were made with an auger of the type manufactured by Iwan Brothers at South Bend, Indiana, by means of which we tried, through

successive sterilization of the auger (before taking each sample), to obtain a sample which represented, uncontaminated, each of the soils as they are found in their natural state in the field. With a sterile spatula there were taken from the samples thus obtained with the auger representative samples which were immediately placed in sterile cotton-stoppered bottles. It was soon found, however, that this method could not be relied upon for accurate results, since no matter how carefully the samples were thus taken, there were many chances for contaminating samples from the lower layers with soil from the upper layers and thus obtaining results which were erroneous. After much experimenting we finally decided on the following plan for taking the soil samples, which, so far as I know, is as free from chances of error as any method that can be adopted in a series of investigations which must of necessity be so extensive. Indeed, I believe the chances for error here are so small that they cannot affect the validity, to any appreciable extent, of the results obtained. Our method consists in having dug, a day or two prior to sampling, a hole twelve feet in depth with at least one vertical wall and large enough for a man to stand in. The samples are taken as follows: With a sterilized spade, a layer of soil of about five or six inches in depth is sliced down along the whole length of the wall which is to be sampled. After this is done, to remove the soil that in the one or two days' exposure may have become contaminated, the fresh surface thus obtained on the vertical wall is sterilized by means of a plumber's torch on the surface surrounding every spot, previously marked off, at which a sample from each foot in depth is to be taken. When this is done a sterile cylindrical tin tube, a little over one inch in diameter and about ten inches long, is driven at right angles to the wall into the spot selected for sampling, immediately drawn out when sufficient soil has thus been obtained, and the cotton plug replaced. In our first experiments, glass tubes of the size described were employed, with paraffined corks at one end and cotton stoppers at the other. We found this to be a poor method, however, and have replaced the glass by tin tubes, closed at one end and plugged with cotton at the other. These are sterilized at 150 degrees centigrade for one hour and a half

before using. In this way by the use of a plumber's torch at every depth as we descend from the surface of the soil down to the twelve-foot depth, we obtain, by starting at sterile surfaces, a sample of soil representing as nearly as possible the true condition which obtains at very depth. The samples are marked properly, taken to the laboratory, and examined for their ammonifying, nitrifying and nitrogen-fixing powers by means of a modified Remy method, the solutions employed for the work being prepared in accordance with the formulae used by J. G. Lipman.[1] Every 50 c.c. portion of the medium in a 250 c.c. Erlenmeyer flask is inoculated with 5 grams of soil.

DESCRIPTION OF SOILS EMPLOYED IN THESE EXPERIMENTS

The descriptions given below represent the soils which were employed for bacteriological examinations and sampled for the purpose as above described. The numbers employed below are used throughout all the following tables so as to make unnecessary any further descriptions.

Soil No. 1. Red clay loam mesa soil, from Riverside, California, on which good orange trees were growing at time of sampling. The soil is well supplied with potash, but rather poor in phosphoric acid and very poor in humus and nitrogen. It is underlaid by hardpan at six feet from the surface, which continues on down to the twelve-foot depth. With the careful cultivation which is given it, along with proper fertilization and tillage, the soil produces profitable crops of oranges and lemons.

Soil No. 2. Silty alluvial loam, from Davis, California. The samples used were obtained from between some fig trees at the University Farm. This soil is practically uniform in color from the first foot to the twelfth and only becomes slightly different in texture below the fifth foot, becoming gradually coarser and sandier as we descend to the lower layers. It is well supplied with potash, phosphoric acid, and lime and has, for a soil of the arid region, a normal content of humus.

Soil No. 3. Sandy alluvial loam, from Davis, California. Samples were taken from a wheat field at the University Farm, only to a depth of ten feet. This soil is well supplied with phosphoric acid, potash and lime, but rather poor in humus and nitrogen. The sand is of a coarse nature and becomes rapidly coarser, descending from the first foot down to the twelfth, where it is found as very coarse sand.

[1] Bulletin 180. N. J. Agr. Expt. Station.

Soil No. 4. Sandy alluvial loam, from Davis, California. Samples obtained in almond orchard at the University Farm. This soil is not nearly so coarse as soil No. 3 and shows a more uniform texture throughout a seven-foot depth, but after that becomes coarser in texture. It is better supplied with humus and nitrogen than soil No. 3 and is well supplied with potash, phosphoric acid, and lime.

Soil No. 5. Alluvial loam, from Davis, California. Samples obtained in a pear orchard at the University Farm. The soil is uniformly of a fine sandy loam texture for a depth of nearly five feet and then rapidly becomes much coarser than the soil at similar depths in No. 3. The upper soil is well supplied with potash, phosphoric acid, and lime, and fairly well supplied with humus and nitrogen. The lower layers are rather poor in phosphoric acid, humus and nitrogen.

Soil No. 6. Fine silty soil, from Hanford, California. Samples taken from a vineyard at Hanford, from the first to the ninth foot only. No sampling was done below the ninth foot because of the fact that the water-table was reached at about that point and it was almost impossible to get samples uncontaminated. This soil is almost devoid of humus and contains but little nitrogen, but is fairly well supplied with phosphoric acid, potash, and lime. No alkali is present in the soil.

Soil No. 7. Silty alluvial loam, from Davis, California. Samples taken at the University Farm, close to a young eucalyptus tree, about twenty months old. The soil is fairly uniform in texture throughout the entire depth studied and is fairly well supplied with humus and nitrogen, and well supplied with phosphoric acid, potash, and lime. No alkali is present.

Soil No. 8. Alkali soil from Tulare, California. Taken only to a depth of ten feet, owing to water conditions such as those described in soil No. 6. This soil contains very little humus and is strongly impregnated with salts, especially ''black alkali.'' It is otherwise well supplied with phosphoric acid, potash, lime, and the other minerals. Hardly any vegetation can exist on this soil after the salts have risen to the upper layers.

Soil No. 9. A very stiff and tenacious silty clay adobe, from Imperial, California. Uniform in texture from the surface down to the eighth foot, at which there is found a layer of fine sand for a foot and a half in depth and then a silty sand below to the twelve-foot depth. The soil throughout is almost devoid of humus and contains but very little nitrogen. It is very rich, however, in phosphoric acid, potash, and lime. The upper layers of the soil consist of particles of silt and clay which are so fine as to become cemented together into an extremely hard, refractory material, which is almost of the consistency of a dry, but not heated, brick. A considerable quantity of common salt is present in this soil. This soil has never been cultivated or cropped.

Soil No. 10. Fine, sandy soil from the desert of Coachella Valley. In this, as in the Imperial Valley soils, there are to be found narrow layers of an inch or two, and sometimes more, of very fine shells of former life which existed in the water at one time covering this land. The soil has very

little or no humus and nitrogen. It is, however, rich in phosphoric acid and lime and well supplied with potash. The soil is uniform in texture throughout the twelve-foot depth and becomes only a little coarser at twelve feet. The only changes visible in color and texture in the vertical wall are merely those of the shell layers above noted. The soil from which these samples were taken has never been cropped, but similar soil, with a good water supply, produces very fine alfalfa. Very little alkali is present in this soil.

Soil No. 11. Fertile, alluvial loam from Hayward, California. Uniform in texture for seven feet and then rapidly becoming quite coarse and remaining so down to a depth of twelve feet. This soil is very fertile, producing good crops of cherries, walnuts, potatoes, and other agricultural plants. It is well supplied with humus and nitrogen, judged by the standard for soils of the arid region, throughout the twelve-foot depth. The phosphoric acid, potash, and lime are also plentiful in all the soil layers. The samples used in these investigations were obtained in a cherry orchard.

AMMONIFICATION IN SOIL COLUMNS

Second only to the importance of soil bacteria in maintaining the total nitrogen supply in soils is their power to supply constantly available nitrogen to plants. The essential nature of this important phase of the activities of soil organisms is in no wise detracted from by the recent research which has made it clear that some plants at least can take their nitrogen from the soil in forms other than the nitrate. While many of them may not absorb their nitrogen in the form of nitrates, it seems quite certain that practically all of them must take their nitrogen in forms much simpler than the proteid. This being undeniably the case, some agency in the soil is necessary to accomplish the transformation of the organic nitrogen (no matter what the source of the latter to the soil may be) into a simpler, more available, or more assimilable form. These agencies we have found to be the various types of soil organisms which constitute what we now designate by the term "ammonifying flora" of the soil.

With these statements admitted, it seems reasonable to suppose that any increase in the activities of the organisms, included under this head, is a distinct advantage to the plant. Under our climatic conditions, where, as above stated, the plant roots very deeply, besides making a large lateral root-development, it is necessary to have the activities of the ammonifying organisms

not only in the upper layers of the soil, but in the lower layers where an actual examination of the root-systems of plants shows a large development of fibrous or feeding roots. A study, therefore, of the ammonifying powers of the different layers of soil, or, rather, of the microorganic flora which they contain, is of practical moment, since it is bound to throw light on the soluble nitrogen supply for roots in the greater depths of soil and indicate what practical measures may be taken toward sustaining and encouraging the growth and activities of the organisms responsible for that soluble nitrogen supply. Since, therefore, we assume ammonia production to be the first great step recognized by our analytical methods in the transformation of soil nitrogen, I have first determined the ammonifying powers at various depths of soils, which may be considered typical of well-defined areas and conditions in the arid region.

For this purpose there were inoculated into sterile 50 c.c. portions of 1 per cent peptone solution, 5 grams of soil from every foot from the surface down to the last depth taken, as above described. After four days incubation at about 28 degrees centigrade, the cultures were washed into copper distilling flasks, sufficient distilled water added, as well as a slight excess of magnesia, and distilled. The distillate was caught in standard tenth normal hydrochloric acid, the excess of which was titrated with standard tenth normal ammonia. Table I gives the results of determinations of the ammonifying power of the soils chosen, as above described. The ammonifying power of only one soil, namely No. 6, is not given, for the reason that the soil column had inadvertently become contaminated before we were ready to use it.

The numbers of the soils refer to corresponding numbers under the descriptions given above, and the amounts of ammonia produced, as given in the table, represent milligrams of nitrogen as ammonia.

The data given in table I prove very clearly two facts. First, that in the typical deep and normal soils of the arid region, the activities and the distribution of the ammonifying flora seem to run parallel with the texture, the chemical composition, and the root-development in these soils. Second, that in the absence of

TABLE I

AMMONIFICATION IN SOIL COLUMNS

Soil No.	1	2	3	4	5	6	7	8	9	10	11
	mg.	mg.	mg.	mg.	mg.		mg.	mg.	mg.	mg.	mg.
1st ft.	59.80	67.76	68.25	61.60	54.67		72.05	7.28	25.46	12.15	42.84
2nd ft.	55.46	77.00	72.66	54.46	49.14		68.90	4.90	8.27	6.46	36.84
3rd ft.	52.30	69.02	68.40	38.50	32.76		70.43	5.46	8.20	3.21	14.14
4th ft.	55.83	70.63	43.05	44.80	lost		65.72	2.80	8.69	1.99	7.28
5th ft.	49.72	67.48	34.23	39.90	8.40		63.69	4.76	4.87	1.25	7.84
6th ft.	49.00	84.63	31.64	47.60	15.68		60.50	4.41	8.27	1.61	21.19
7th ft.	31.70	72.17	42.00	44.80	17.85		56.90	2.45	6.03	1.57	7.14
8th ft.	30.54	52.57	35.00	22.54	9.80		50.43	10.22	5.40	1.34	7.48
9th ft.	28.65	52.43	35.70	32.90	15.40		45.69	10.64	2.18	1.24	5.60
10th ft.	25.40	36.89	....	29.40	10.22		43.25	....	2.23	2.11	16.38
11th ft.	15.65	21.56	....	25.48	11.06		40.76	....	1.34	4.87	16.64
12th ft.	14.30	35.84	....	38.22	10.50		38.45	....	2.62	1.44	10.85

humus and moisture, or in the presence of alkali salts, the activities of ammonifying organisms are seriously handicapped.

To discuss these more in detail we find, for example, in soil No. 1, derived from the mesa soil at Arlington Heights, Riverside, a strong ammonification, varying but little from the first foot down to the seventh, below which depth we find a sudden marked decrease in ammonia production, for the reason, doubtless, that from the sixth foot down to the twelfth we find a layer of hardpan which, owing to its poor aëration and poor water conditions, is unfit for the development of a vigorous bacterial flora. In other words, we find in this soil-column, through the ammonifying power of the various depths of soil, an expression of the vigor and numbers of bacteria present in these soil layers and also of the amounts of soluble nitrogen which can there be expected to be made available through the agency of soil organisms.

In soil No. 2, however, which represents a good, deep alluvial soil, we find a very vigorous ammonification from the first seven feet, and only slightly reduced ammonia production in the eighth and ninth feet, after which we find a large reduction of about 50 per cent in ammonia production for the other three feet. We have here, therefore, good vigorous ammonia production down

to the tenth foot and therefore an indication that in these soils there is constantly being made available nitrogen, if organic nitrogen be present from humus and other sources, for the needs of plants with deep root systems.

In soil No. 3, which is more sandy than the other alluvial soil described and which rapidly becomes coarser in texture as we descend into the lower layers, we find vigorous ammonification to obtain down to the fourth foot, below which we find a considerable decrease in ammonifying power, owing to the fact that in that coarse soil neither water nor humus, nor soluble minerals, are present in sufficient quantity to encourage bacterial development. Here, however, we find the general tendency for ammonifying organisms to penetrate to the greater depth quite plainly visible. The remarks made for soils 2 and 3 are just as truly applicable to the other alluvial soils from the same district represented by Nos. 4, 5, and 7. The marked production of ammonia, even in the twelfth foot of No. 7, is in accord with the fine physical and chemical condition of that soil to that depth and therefore deserves additional mention here.

As to other types of soils, the data in the table show plainly enough what a profound effect strong alkali salts (both black and white alkali salts among them) may exert on the ammonifying flora and their vigor. Here ammonification is indeed very feeble in the surface soil, becoming feebler as we go down until the eighth foot is reached, at which depth, as well as in the ninth foot, we find quite a marked increase in ammonia production. This is doubtless due to the fact that the total salt-content is at that depth much lower and therefore not so seriously affecting the activities of the organisms there contained. As for the desert soils, which never have contained much humus and very frequently contain too much alkali, it is natural to expect a rather feeble ammonifying power on the part of the soils. Table I shows that in this case the expected happens. In soil No. 9, for example, not only the lack of humus and moisture, but the very unfavorable physical condition, above referred to under the description of that soil, along with its salt-content, have so far affected the ammonifying power of that soil as to reduce it to a little over one-third of what the normal valley soils de-

scribed have exhibited. Moreover, it would seem that the salt-content in the lower layers of this soil, which increases as we go down, has very seriously checked the development of these organisms there and was probably assisted by the unfavorable physical condition mentioned. In soil No. 10, while the salt-content is only meager, we have a rather coarse, sandy soil with hardly any humus, which is therefore for that reason an unfavorable medium for the development of bacteria, to say nothing of the lack of moisture there and the great heat which these desert soils must absorb from the sun. We therefore have a very much smaller ammonifying power in the upper layers of the soil than exists even in soil No. 9, from Imperial, and then a very rapid decrease to almost no ammonifying power in the lower layers.

By a general survey of all of these data, it would certainly seem that we are justified in drawing the conclusion that ammonification and the ammonifying flora of soils are vigorous for several feet down in the arid region and are limited in their activities only by the presence of large amounts of salt or a lack of humus and moisture. Since, however, California soils, taken by and large, are deep, we have reason, from the facts above given, to suppose that the ammonifying power in most of these soils, which are not in any way "abnormal," is vigorous at great depths.

NITRIFYING POWERS OF SOIL COLUMNS

By very many and perhaps by most plants, nitrate is the form of nitrogen taken up. It is therefore of importance not only to study ammonia formation in soils, but nitrate formation as well. In these investigations we have studied qualitatively and quantitatively the production of nitrites and nitrates in ammonium sulfate solution by soils from the different depths in every case. Here also, as in the ammonification work, 5 grams of soil were used to inoculate 50 c.c. of culture solution. The results obtained in this work are set forth in a qualitative manner, as to nitrate formation merely, in table II, since it is sufficient for the purpose of this preliminary paper to know to what depths in the soil nitrates are produced. Later publications, giving the more complete data of these investigations, will

give the quantitative results as they are given for ammonification in table I. The plus sign represents nitrate formation and the minus sign the absence thereof. The numbers of the soils are referred again to the descriptions above given.

TABLE II

NITRIFICATION IN SOIL COLUMNS

Soil No.	1	2	3	4	5	6	7	8	9	10	11
1st ft.	+	+	+	+	+	+	+	—	—	—	+
2nd ft.	+	+	+	+	+	—	+	—	—	—	+
3rd ft.	+	+	+	+	+	—	+	—	—	—	+
4th ft.	+	+	+	+	+	—	+	—	—	—	+
5th ft.	+	+	+	+	+	—	—	—	—	—	+
6th ft.	trace	+	—	—	+	—	—	—	—	—	+
7th ft.	—	—	—	—	—	—	—	—	—	—	+
8th ft.	—	—	—	—	—	—	—	—	—	—	+
9th ft.	—	—	—	—	—		—		—	—	—
10th ft.	—	—		—	—		—		—	—	—
11th ft.	—	—		—	—		—		—	—	—
12th ft.	—	—		—	—		—		—	—	—

From the data in table II we see again a striking resemblance between nitrification in the soil depths and ammonification in the same. All the alluvial soils in particular show very uniform nitrate formation. The latter seems to be as much inhibited in soil No. 1 by the hardpan layer as is ammonification. In soils 2, 3, 4, and 5, as well as 7, we find a general tendency for nitrates to be formed in the first five feet and then an enfeebled power of nitrate formation, in some cases for one foot and in other cases a total loss of that power. In nearly all cases these run parallel with a similar decrease in ammonia formation, but it seems that nitrate formation is more seriously hampered by the conditions which curtail ammonia formation and particularly, it appears, by the lack of oxygen in the lower layers of the soil. This is an account, therefore, of the first case which has come to my notice of nitrification at any depths below two or three feet in the soil, and shows a marked difference in itself between soils formed and existing under humid and those formed and existing under arid conditions. Nitrogen therefore is available in

these soils, not only for those plants which are able to absorb ammonia nitrogen, but also for that larger class of normal plants which absorb their nitrogen in the nitrate form. It must be said here, however, that nitrate formation proceeded always more rapidly in soils from the first foot than in cultures prepared from the other depths. This may indicate a smaller number of nitrifying organisms in the lower layers of the soil or perhaps a less vigorous flora, but their activities are uniform from the second foot down to the last depth in which they show no activity as indicated in table II. In soil No. 6, which we find on analysis contained merely a trace of humus, that circumstance seems to have made the soil unfit for the development of the vigorous bacterial flora and is supported by the data in tables II and III. As to the nitrate formation in culture solutions by the inoculation with this Hanford soil, nitrates were produced only after a month's incubation and only in small quantities in the culture prepared from the upper foot of soil, whereas all other surface soils, when inoculated into solutions with the exception of those which show no nitrification at all, showed nitrate formation before the end of two weeks. In culture solutions from soil No. 6, kept about three months and prepared from the lower layers of soil, no nitrates were ever to be found. The depressing effect of alkali on the bacterial flora, as well as the inhibiting effect of a lack of humus, moisture, and the proper physical condition, are again exemplified in table II in soils 8, 9, and 10, as they were for the same soils in table I referring to ammonification. Even after one month's incubation, not one of these soil-samples showed any nitrate formation, whether the culture was prepared with the soil from the upper layers or from the lower. There seems to be a total absence of nitrifying bacteria of one kind or another.

The best example of the penetration of nitrifying bacteria to great depths was obtained in soil No. 11, a fine alluvial loam from Hayward, where nitrate formation was obtained down to the ninth foot in the soil. In this case also there was, besides a mere formation of nitrates, as shown by a qualitative test, an actually vigorous nitrate formation in the lower layers as well as in the upper layers of the soil. It would seem again here

therefore, in general, that where soils in the arid region are supplied with a moderate amount of humus, with the proper texture and chemical constitution, as well as freedom from alkali, all of which is true of the large majority of our soils, nitrification as well as ammonification is found to obtain vigorously in the lower layers of the soil for four feet at least, and in some cases to six and to nine foot depths.

NITROGEN FIXATION IN SOIL COLUMNS

The next point of interest to determine in these soil-column investigations from the bacteriological standpoint was to show whether or not the supply of nitrogen, at the disposal of the ammonia-forming and nitrate-forming organisms, which we have found developed to such great depths, and enabling roots to have a soluble nitrogen supply there, was provided merely by the humus content of the soil at those depths and produced from decaying roots, or carried down from the upper layers; or, was that nitrogen supply in part a new one obtained directly from the atmosphere by nitrogen-fixing bacteria. If such were the case, we should, of course, have enormous quantities of nitrogen fixed per acre, since the fixation would not be limited to the upper foot of soil. Accordingly, experiments were inaugurated to obtain the facts which exist with reference to this matter.

Here the necessary mannite solution was inoculated with five grams of soil in each case, and a culture prepared from every foot in depth in the case of every soil. Table III shows in tabular form the results obtained, which are set forth qualitatively. The numbers at the heads of the columns refer again to the numbers used in the description of soils, and one plus sign is intended to show the presence of Azotobacter, two of a fairly vigorous development of these organisms, and three of a very vigorous development. In this qualitative way, therefore, nitrogen fixation has been judged by the development of Azotobacter as a criterion. It may justly be argued against this that other organisms are capable of fixing nitrogen and that the quantitative figures would be preferable to the qualitative one showing merely the presence of Azotobacter. While this argument may in part be true, it appears from my results, which

show quantitative as well as qualitative figures in these as well as other experiments, that in the absence of Azotobacter only very slight fixations of nitrogen or none are obtained.

From the results set forth in table III, it appears that only one soil of the eleven tested shows the presence of Azotobacter as deep down as the fourth foot and six others show the presence of these organisms in the third foot. Most of them, however, show the presence of Azotobacter in vigorous form only in the first two feet. It is therefore not sufficient, evidently, for

TABLE III

NITROGEN FIXATION IN SOIL COLUMNS

Soil No.	1	2	3	4	5	6	7	8	9	10	11
1st ft.	+++	+++	+++	+++	+++	—	+++	—	—	—	++
2nd ft.	++	+++	+++	+++	+++	—	+++	—	—	—	++
3rd ft.	+	—	++	+++	+++	—	++	—	—	—	++
4th ft.	—	—	—	—	+++	—	—	—	—	—	+
5th ft.	—	—	—	—	—	—	—	—	—	—	—
6th ft.	—	—	—	—	—	—	—	—	—	—	—
7th ft.	—	—	—	—	—	—	—	—	—	—	—
8th ft.	—	—	—	—	—	—	—	—	—	—	—
9th ft.	—	—	—	—	—	—	—	—	—	—	—
10th ft.	—	—	—	—	—	—	—	—	—	—	—
11th ft.	—	—	—	—	—	—	—	—	—	—	—
12th ft.	—	—	—	—	—	—	—	—	—	—	—

soils to be chemically and physically as favorably constituted as these soils are for ammonification and nitrification to encourage the deeper penetration of Azotobacter. As is well known, these organisms are extremely sensitive to a lack of oxygen and it would appear that this circumstance regulates and controls the penetration of Azotobacter organisms as above portrayed. I think in addition, however, it may fairly be argued that the presence of Azotobacter in more than half of these soils in the third foot is, in itself, a favorable indication of the nature of the soils in question. It is of interest also that, in soil No. 5, Azotobacter organisms, with the nitrogen-fixing power as vigorous as those above, were found in the fourth foot. This, so far as the writer is

aware, constitutes the only published case of even this extent of penetration of Azotobacter organisms. It has been reported to me, however, that Azobacter organisms have been found in the twelfth foot of soil in some of the very favorably constituted loess soils of Nebraska. The question put in the introduction to this subject of nitrogen fixation is therefore answered in the negative. For the greater depths, at any rate, in which ammonification manifestly is vigorous in our soils, Azotobacter organisms do not penetrate and are not the source of the supply of nitrogen which can be transformed at those depths by the ammonifying organisms or by the nitrifying organisms. The nitrogen supply of these, therefore, in the lower layers of the soil must be the humus produced from the decaying roots at those depths, or the humus brought down in solution from the upper layers of the soil.

As regards soil No. 6 we have here again a total absence of Azotobacter organisms, possibly due in part, at least, if not wholly, to the absence of any but very small amounts of humus, by which I have already tried to explain the feeble nitrification only in the first foot of this same soil. The same remarks also which were made above, with reference to soil Nos. 8, 9, and 10, as regards their ammonifying and particularly their nitrifying power, apply again in the case of their nitrogen-fixing power. No Azotobacter organisms and no fixation of nitrogen were ever observed in any of these soils, no matter from what depth of soil the cultures were prepared.

In justice to this subject it must further be stated here that the comparatively slight penetration of Azotobacter organisms in our soils may be due to factors other than merely a lack of a plentiful supply of oxygen. There is evidently some other circumstance which controls the presence or absence in many of our soils of Azotobacter organisms and that may also limit the depth to which these organisms may penetrate. Just what this factor may be is not at present clear to the writer, but the fact remains that frequently soils with a good chemical and physical constitution and producing good crops, will yet show no Azotobacter organisms.

GENERAL DISCUSSION

As I have already pointed out in an earlier publication,[2] the slow formation of clay substances in soils of the arid region, owing to the peculiar climatic conditions there obtaining, is doubtless responsible for a much greater degree of aëration in soils because of the larger volume of pore spaces made possible through a lack of large quantities of cementing substances. Thus when soils first begin to form from disintegrating rock we have much more complete aëration with an encouragement for bacteria, probably the earliest inhabitants of the soil, to penetrate to greater depths. Such penetration on the part of bacteria is invariably accompanied by the production of more favorable physical and chemical conditions in the soil for the roots of plants. These in their turn, through physical and chemical changes which they bring about in the soil in their search for water and food, make better conditions for a deeper penetration of bacteria and so through mutual aid the latter and the higher plants are able, under our arid climatic conditions, to make the deeper layers of soil a more congenial medium for each other. The changes thus brought about result in a more uniform texture of soils at great depths, uniformity of chemical composition, including humus content, in all the soil layers, and a much closer approximation of the bacterial flora in the lower soil layers to those of the upper layers than can be found in the average soils of the humid region, where climatic conditions are unfavorable to good aëration, because tendencies opposite to those above described for our soils are in operation. An estimate of the biological condition of our deep soils was thus similarly made by Hilgard on *á priori* considerations and the investigations above recorded serve, in general, to confirm his surmise.

Viewing the subject in its entirety, we find that the organisms forming ammonia in soils penetrate to greater depths than the nitrifying or nitrogen-fixing bacteria studied. While ammonification is usually most vigorous in the surface, four to six feet, it is none the less very pronounced in the lower layers from six to ten feet in depth in all of our normal deep soils. Hardpan,

[2] Lipman, C. B., New Facts about Bacteria of California Soils, Science N. S., June 11, 1909.

alkali, and a lack of humus and moisture decrease the ammonifying powers of our soils or are not favorable to the development of vigorous ammonifying flora, but their effects are just as pronounced in the upper layers of these abnormal soils as in the lower layers which, therefore, cannot be fairly compared with our deep average soils as to bacterial content. To what a serious extent alkali salts may affect ammonification has been shown by me in a recent paper.[3] That the humus content alone may profoundly affect the number and vigor of bacteria is well exemplified in both soils No. 6 and 10, where all other conditions but the humus content are favorable and where both the number and physiological efficiency of the organisms is small.

It would therefore seem, in brief, that ammonification is vigorously active in the lower soil layers in soils of the arid region where humus is present and hardpan and alkali are absent. Since these conditions are complied with in the average of our cropped soils, the opinion is justified that the deep penetration of bacteria is a distinctive characteristic of soils in arid regions which results from much better aëration, as a starting point, than can be attained in soils of the humid region. The experimental data above given amply confirm this opinion and help to explain why deep plowing is not only harmless in our soils but directly beneficial, and why three or four feet of upper soil may be removed in grading, and alfalfa and fruit trees may be grown on the newly uncovered subsoil without difficulty, a feat which cannot be accomplished on soils of the humid regions.

As for nitrification my data present again features of striking interest. They go to prove that nitrate formation, like ammonification, goes on at much greater depths in soils of arid than in soils of the humid region, and thus render distinctly sectional the observations of Dyer[4] on this subject, and makes them applicable only to soils of the humid region. While the nitrifying organisms are doubtless more susceptible to a lack of oxygen than the ammonifying bacteria, the differences obtained above between the two groups of organisms, so far as soil fertility is concerned, are rather those of degree than of kind. The same relationships

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[4] Bulletin 106, p. 55, O. E. S., U. S. D. A.

[3] Centrallblatt für Bakt., 2 Abt., vol. 32, p. 58.

displayed by the ammonifying bacteria toward hardpan, alkali, and a lack of humus and moisture, hold in a more exaggerated way as regards the nitrifying organisms. More specifically, the writer has also shown[5] the distinct effects of each of the alkali salts on nitrifying bacteria in work quite recently completed. It would appear in general, however, that in our deep soils, a supply of nitrate as well as of ammonia is at the disposal of plants for a depth of five or six feet. As regards the nitrogen-fixing powers of soils of the arid region, my results show plainly that they do not differ strikingly from those of soils in the humid regions, if the presence and vigor of Azotobacter organisms be taken as a criterion. While it is true that in one or two cases Azotobacter organisms were found in our soil-columns below the depth at which they occur elsewhere, and perhaps at a slightly greater depth in all soils in which they were found, I feel loath to believe that these are expressions of a rule for soils of the arid region. Other observations indeed lead me to believe that Azotobacter development has not gone so far in our soils as it has in soils of other regions. For example, I have studied many soils in California with a favorable physical and chemical constitution which were absolutely devoid of Azotobacter organisms. If therefore the results set forth above, with reference to nitrogen fixation, are to be considered representative, the nitrogen supply in the lower layers of the soil must be replenished in this region as well as in the humid region, not from direct fixation by Azotobacter, but from the nitrogen of the upper soil layers.

With reference to these investigations in general, one or two additional points need more than passing consideration. First, as to the method of collecting the soil-samples for examination, it appears to the writer that every possible precaution was used to prevent contamination and it would be difficult to devise a method which takes into consideration and avoids more of the avenues of contamination by which any results might be vitiated. Moreover, I find strong confirmation of this belief in the facts brought out in the data above given, viz., that any abnormality in the soil was sure to be reflected in the results obtained with cultures prepared from that abnormal soil. Thus hardpan layers

[5] Cent. für Bakt., 2 Abt., vol. 33, p. 305.

never gave evidence of vigorous bacteria, nor did alkali soils or soils devoid of humus.

Secondly, the writer desires to anticipate criticism on the method used in culturing the organisms of the various soil samples, viz., a modified Remy solution method. No one is more ready than I am to admit the just criticism made of the solution-culture methods in soil bacteriology. Indeed I believe that I was one of the first to put into practice on a large scale the *direct* soil-culture method in the laboratory. But when problems of the nature involved in these investigations must be attacked, regard must be had for the chances of contamination in the method employed, and for the feasibility of obtaining, uncontaminated, large volumes of soil for use in these experiments. When these were considered from all points of view, only one feasible and reliable method of culturing the soils seemed available and that was the solution method. The difficulties, practically insurmountable, which must arise with any other method, when such work is carried out on a large scale as it must of necessity be, can be fully appreciated by those who have ever attempted it. The gratifying results obtained in this work, however, seem to me a further justification of the methods employed.

It seems of particular moment now, to call the attention of soil bacteriologists in particular, and soil scientists in general, to the important field explored in these investigations and the striking results obtained therefrom, not only because it represents a new field of research, but because it emphasizes more strongly than ever the radical differences which obtain between soils of the humid and arid regions. It also helps to explain the extraordinary appearance of our subsoils (if subsoils they be) and the marvellous root developments of which plants under our climatic conditions are capable. While these studies have not yet departed from the realms of the preliminary, they are replete with facts which are already of considerable practical and scientific significance and which are doubtless destined to become more so as time progresses. As a part especially of a comprehensive soil study they are invested with unusual importance and may help to solve problems now perplexing and difficult to study.

CONCLUSIONS

Investigations of the distribution and activities of bacteria in soils of the arid region show:

1. That samples of soil for studying the flora of each layer of soil can best be obtained from a hole twelve feet in depth with at least one vertical wall, the latter when sterilized being sampled.

2. That tin tubes ten inches long and about one inch in diameter closed at one end and cotton-stoppered are best for collecting the samples.

3. That the solution method for studying the soils, despite its many drawbacks, is the most feasible one to employ.

4. That soils of the arid region at all depths studied show ammonifying powers which, however, are generally most vigorous in the first six or eight feet. In one case ammonification was noted in soil from a depth of fifteen feet, or adjoining the water-table.

5. That nitrification is found commonly down to a depth of five to six feet in soils of the arid region. In one case soil from the eight-foot depth showed a vigorous nitrifying power.

6. That nitrogen fixation through Azotobatcer does not go on below two feet in the soil usually, but has been found in some soils at three feet and in one soil down to four feet. Many soils in the arid region, otherwise favorably constituted, do not contain Azotobacter organisms.

7. That from the point of view of ammonification and nitrification soils in the arid region differ markedly from those in the humid region when the lower layers of soil are considered. The difference is not marked as regards nitrogen fixation.

8. The results above recorded help to explain the favorable physical and chemical constitution of our soil and also the deep rooting of plants so characteristic of the arid regions.

Transmitted April 8, 1912.